AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

STATUTORY DECLARATION

I, Peter Adrian Walton Rogers of the Department of Obstetrics and Gynaecology, Monash University, Clayton, Victoria, Australia, do solemnly and sincerely declare as follows:

I. INTRODUCTION

1.1 Ludwig Institute for Cancer Research ("Ludwig Institute") has asked for my services as a scientific expert in connection with Ludwig Institute's opposition to the issuance of an Australian patent to Human Genome Sciences, Inc., ("HGS") based on HGS's Australian Patent Application No. 696764. The patent application relates generally to an isolated polynucleotide and protein for an alleged novel vascular endothelial growth factor called "Vascular Endothelial Growth Factor 2" ("VEGF2").

1.2 The first evidence that I provided in the opposition proceeding was a declaration from February 2000 (hereinafter referred to as "OPR1" (Opponent, Peter Rogers, 1st Declaration)). I am aware that Ludwig Institute has also asked Francis John Ballard and Kari Alitalo to serve as scientific experts in this matter, and that they have provided declarations as well.

1.3 In answer to Ludwig Institute's initial evidentiary submission, HGS filed declarations from six scientists. John Stanley Mattick (hereinafter "AJM1"(Applicant, John Mattick, 1st Declaration)), Jennifer Ruth Gamble ("AJG1"), Nicholas Kim Hayward ("ANH1"), Thomas Rapoport ("ATR1"), Stuart Aaronson ("ASA1"), and Susan Power ("ASP1").

1.4 In reply to HGS's declarations, Ludwig filed second declarations from Dr. Alitalo (OKA2), Dr. Ballard (OJB2), and me (OPR2). HGS then obtained permission to file still further evidence, in the form of supplemental declarations by Dr. Power (ASP2), Dr. Hayward (ANH2), and Dr. Aaronson (ASA2). Ludwig Institute asked me to review these most recent declarations filed by HGS and evaluate them in the context of all of the documents that have been filed in this proceeding. Ludwig Institute has also asked me to review declarations of Dr. Ballard and Dr. Alitalo that were prepared in response to ASP2, ANH2, and ASA2.

1.5 I have reviewed all of the foregoing declarations, including the third declarations of Drs. Ballard and Alitalo (OJB3 and OKA3) that were filed in July, 2002, and the fourth declaration of Dr. Alitalo (OKA4) that I understand will be filed in August, 2002.

1.6 I hereby reaffirm my understanding that I have an overriding duty to the Patent Office (and to any Australian Federal Court that should review the Patent Office decision) to provide objective scientific analysis that I believe to be truthful. I hereby affirm that, to the best of my knowledge and belief, factual statements herein are true and opinion statements herein represent my objective scientific opinion and analysis.

II. AFFIRMANCE OF PRIOR DECLARATIONS

2.1 Unless I specifically state otherwise below, I affirm the facts and opinions expressed in my prior declarations (i.e., in OPR1 and OPR2). Nothing in HGS's evidentiary declarations causes me to change the opinions embodied in my earlier declarations.

2.2 In fact, HGS did not specifically dispute my second declaration in the further evidence that they submitted. Whereas my earlier declarations were directed to numerous problems with the opposed application and the HGS evidence in answer, including lack of novelty, inadequate and incomplete description of the invention, hindsight vision by

the HGS declarants, lack of any evidence of VEGF2 biological activity, errors and omissions in the application, and indefinite terminology, the further evidence filed by HGS is primarily directed to the narrower question of the validity and relevance of experiments by Dr. Power and Dr. Alitalo reported in their respective declarations. Therefore, I shall confine most of my remarks herein to those issues, even though I continue to believe that the many problems with the opposed application, enumerated in my earlier declarations, continue to exist.

III. AGREEMENT WITH DR. BALLARD'S THIRD DECLARATION

3.1 I have read the third declaration of Dr. Ballard and I agree with the opinions expressed by Dr. Ballard in his declaration. I hereby incorporate the analysis therein as my own by reference.

IV. THE EXPERIMENTS REPORTED IN DR. ALITALO'S DECLARATIONS ARE SOUND, AND THE CRITICISMS OF THEM ARE MERITLESS.

4.1 I have read all four of the declarations of Dr. Kari Alitalo that were prepared in respect of this matter, including the most recent Alitalo declarations (OKA3 and OKA4) prepared in response to the latest declarations filed by HGS.

4.2 I continue to hold the opinion, expressed in OPR2, that the Alitalo experiments were appropriately designed to evaluate the teachings of the opposed application (OPR2 at 2.2, 2.5). The opposed application teaches that VEGF2 is about 350 amino acids of which approximately the first 24 amino acids are likely to be leader sequence such that the mature protein comprises 326 amino acids. (Opposed application at p. 5. See also Figure 1 and sequences.) Dr. Alitalo's experiments remain the only experimental evidence in this proceeding directed to the question of whether VEGF2, as taught in the opposed application, is expressed and secreted by cells. The opposed application has no evidence of successful expression of mature VEGF2, as discussed extensively in my first declaration (OPR1 at 7.6 and sections concerning Insufficiency and Fair Basis). The experimental evidence offered by Dr. Power is not based on the opposed application, as explained in my second declaration (OPR2 at 2.5), in Dr. Ballard's third declaration (OPR3 at 6.1-7.4), and below in greater detail.

4.3 Through four independent sets of experiments, Dr. Alitalo has repeatedly found and reported that VEGF2 expression and secretion as taught in the application does not occur in cells. His second, third, and fourth sets of experiments included modifications to address criticisms raised by HGS declarants. I agree with Dr. Alitalo and Dr. Ballard that the OKA3 and OKA4 experiments contained appropriate controls for the variables questioned by HGS in its second series of declarations, namely, transfection efficiency and time course.

4.4 As explained repeatedly in Ludwig Institute's evidence and admitted by the HGS declarants in their first series of declarations, the VEGF2 of the opposed application is *incomplete* (OPR2 at 4.1, 4.3-4.6). It is missing about 69 codons, including the codons for the signal sequence. These defects were not reported in the opposed application, but were discovered in subsequent years of research. However, these acknowledged defects provide one scientific explanation for why Dr. Alitalo repeatedly finds that VEGF2 as taught in the opposed application cannot be expressed and secreted. In my opinion, the criticisms raised by HGS through its declarations are little more than a smokescreen to distract from the plain truth that the teachings in the opposed application relating to VEGF2 are incomplete. The lack of substance behind the HGS criticisms is illustrated by the willingness of the HGS declarants to espouse inconsistent positions. (See detailed discussion in OJB3, Section IV.) For example, Dr. Hayward first opined that Dr. Alitalo's experiments were unreliable because of inconsistencies in expression and/or processing by different cell lines used for VEGF2 expression studies. (See ANH1 at 5.5.) After Dr. Alitalo provided additional controls and explained that this criticism was without merit, Dr. Hayward asks the Patent Office to believe that "By March 1994 I was aware that any given host cell would possess the proteolytic enzymes and cellular machinery to naturally process a protein such as VEGF-2 to its mature form." (ANH2 at 1.7) If Dr. Hayward were aware as early as 1994 that any cell would work, then why was he criticizing Dr. Alitalo's choice of cells in his ANH1 declaration?

4.5 Most of HGS's criticisms of Dr. Alitalo's work have been directed to whether his experiments contain adequate controls. With almost any experimental summary, a scientist with general knowledge in a field can identify some parameters for which the experimenter did not report controls. At the same time, such a scientist is also familiar with which aspects of an experiment are routine or uncontroversial, and which are more critical to the variable being tested. In my opinion, none of the controls on which the HGS experts focus are serious experimental omissions, if indeed Dr. Alitalo omitted them at all.

4.6 In any event, it is clear to me from reading OKA3 and OKA4 that Dr. Alitalo has run his experiments twice more, with explicit controls for transfection efficiency and time course, the two factors that were the subject of HGS's newest declarations. Dr. Alitalo's results show that these factors are not affecting his results (OKA3 at 6.1; OKA4 at 6.1). Dr. Alitalo's experiments have always been designed to compare expression of VEGF2 as taught in the opposed application with a true, full length VEGF-C cDNA, which can be viewed as a "positive control." The experiments have also included "mock" transfected cells as a negative control. The only meaningful variable in Dr. Alitalo's experiments was the identity of the insert in the expression vector (VEGF2 or VEGF-C). The VEGF-C "positive control" insert worked just fine, but the VEGF2 as taught in the opposed application failed, demonstrating that cells cannot express and secrete VEGF2 as taught in the opposed application.

V. DR. POWER'S DECLARATION

5.1 Dr. Alitalo and Dr. Ballard have explained in their declarations, and I agree, that the experiments by Dr. Power are not based on the teachings of the opposed application (OJB3 at 6.1-7.4; OKA3 at 2.1). I also agree with their conclusion that her results -- "a broad band resolving at approximately 30 kDa" on a gel (see ASP2 at 31) -- have no apparent relevance to the teachings of the opposed application. (See, e.g., OJB3 at 6.1-7.4; OKA3 at 5.1-5.7.) The opposed application teaches that mature VEGF2 comprises 326 amino acids of the VEGF2 sequence taught in the application. These 326 amino acids have a combined, calculated theoretical molecular weight (for "mature VEGF2") of about 36.8 kD, ignoring the possibility of post-translational modifications such as glycosylation that might increase the observed molecular weight.[1] This calculated weight is in reasonable agreement with the observed molecular weight of the two polypeptides reportedly produced by "in vitro transcription/translation" in Example 2 of the opposed application, which were reportedly 38-40 kD for "the full length VEGF2 cDNA" and 36-38 kD for a polypeptide reportedly missing 36 amino acids from the C terminus. The 30 kD molecular weight reported by Dr. Power in

[1] Of course, the notion of an observed molecular weight is theoretical only, since that would require cells to express and secrete mature VEGF2 as taught in the application, and Dr. Alitalo has shown that this does not happen.

her experiments has no apparent relevance to the teachings in the opposed application regarding VEGF2.

5.2 To explain this discrepancy in a different way, I would say that the skilled person, in attempting to express VEGF2 as taught in the opposed application, would have been looking for a polypeptide of approximately the full length as taught by HGS. That person would have expected to produce a mature VEGF2 of about 37 kD based on calculated molecular weight and based on the limited guidance provided by Example 2 of the opposed application. For these and other reasons, it is my opinion that Dr. Power's experiments should not be accepted as relevant to the opposed application. Even if, for argument's sake, Dr Power's results are accepted for some purpose, I do not believe that a scientist in 1994, without the benefit of what is now known about VEGF-C, would have identified anything other than the 326 amino acid "mature VEGF2" polypeptide from the application itself, and would not have had any reason to investigate anything other than the 326 amino acid polypeptide.

5.3 Dr. Power's experimental approach to solving defects in the opposed application was clearly developed with the benefit of hindsight. (See, e.g., OPR2 at 4.24 and 4.54-4.71.) Dr. Alitalo's work shows that a scientist in 1994 attempting to express the VEGF2 sequence by following the teachings of the opposed application would have been unsuccessful, e.g., because as extensively explained in the Ludwig Institute's evidence and sometimes admitted by HGS declarants, the VEGF2 of the opposed application is incomplete. (OPR2 at 4.1 and 4.3-4.6) It is missing about 69 codons, including the codons for the signal sequence. That scientist would have then needed further experimentation to identify the source of the problem or problems and determining the solution or solutions to those problems. However, without the benefit of hindsight that we now enjoy, that scientist would not have known that the failure was due, at least in part, to a missing signal peptide and other N-terminal sequences.

5.4 The absence of a signal sequence would not, in my opinion, have been the only possibility nor would it have been the first possibility as to the source of the problem. In fact, I believe the scientist would have been comforted and guided by the express statements in the opposed application that the VEGF2 sequence as taught therein includes a signal sequence, and so would have concentrated on the numerous other possible problems in expressing a novel, as yet uncharacterized human cDNA. These possibilities

include problems associated with the vector (such as absence of controlling elements such as promoter sequence, enhancer sequence), problems associated with the host cells (such as inadequate growth and proteolysis), problems of incompatibility of the host cells and the vector or the encoded protein, problems associated with the induction of expression (such as inactive or insufficient inducing agent), problems associated with the coding sequence (such as the presence of premature termination codon), problems associated with the detection system (such as low titre of an antibody for antibody detection, lack of activity for an activity-based detection, defective label for label-based detection), problems associated with degradation, and so on. I discussed these issues extensively in my second declaration. (See OPR2, sections regarding Fair Basis and Insufficiency.)

5.5 It is interesting to observe that when HGS discovered that the complete VEGF2 cDNA contains approximately an additional 69 codons, meaning that VEGF2 as taught in the opposed application is incomplete, HGS considered the discovery to be significant enough to file a second series of patent applications on the 419 amino acid form of VEGF2. (See Documents D44-D46; OPR1 at 1.5.1-1.5.2). In the second series of applications, HGS teaches that the immature and "mature" forms of VEGF2 are substantially longer than what was taught in the opposed application (419 and 396 amino acids, respectively).)See Documents D43 and D44 at p. 7, last paragraph; OPR1 at 4.11.1.1.) Of course, in this opposition proceeding, HGS and/or Dr. Power can design some experiments focused directly on the problem of the missing signal peptide, but only through the benefit of hindsight knowledge that VEGF2 as taught in the opposed application is missing the signal peptide.

VI. DR. ALITALO'S "FAILURE" TO USE A HETEROLOGOUS SIGNAL SEQUENCE.

6.1 Drs. Hayward and Aaronson argued that Dr. Alitalo's experiments are flawed because in Dr. Alitalo's declarations, he did not report on the effect of attaching a heterologous signal sequence to the 350 amino acid VEGF2 sequence. (See, e.g., ANH2 at 1.5, 1.6, 1.10; ASA2 at 5, 6-10, 13-22.) Dr. Power's experiments involve heterologous signal peptides.

6.2 As explained in the preceding section and by Dr. Ballard in his third declaration (e.g., OJB3 at 6.1-7.4), I disagree with the Hayward/Aaronson analysis and

disagree with any suggestion that Dr. Power's experimental approach is a fair representation of anything taught in the opposed application. The opposed application teaches that VEGF2 already has a signal sequence. (See, e.g., opposed application at p. 5, last paragraph.) It is illogical to attach a heterologous signal sequence to a sequence which already has a signal sequence. Solving the riddle of VEGF2 expression would have required a scientist to ignore the specific teachings regarding VEGF2 that are found in the opposed application and arrive at a solution that is completely at odds with those teachings. As extensively explained in Ludwig Institute's second evidentiary submission, the opposed application does not teach to perform the experiments urged by HGS. [OJB2 at 2.2, 3.19-3.24, 6.1-6.6.; OPR2 at 4.4-4.6, 4.12, 4.14-4.53.]

6.3 In my opinion, Dr. Alitalo's experiments were properly designed to test the opposed application's teachings, which are that full length VEGF2 comprises 350 amino acids, of which approximately the first 24 amino acids are a signal sequence. (See, e.g., opposed application at pp. 4-5). These were the specific teachings to the public about the subject matter of the opposed application. The experiments urged by HGS are a form of revisionist history - an attempt to fix a defective patent application by hindsight knowledge of a protein learned from publications by Dr. Alitalo and others. [See, e.g., Documents D70-D73.] The question as I understand it is not whether a person in year 2001 or 2002, knowing what Dr. Alitalo and others have taught the public about the VEGF-C gene and protein, can apply that knowledge and succeed at expressing some portion of the VEGF2 sequence taught in HGS's 1994 patent application. The question is whether the teachings of the application were accurate and placed the public in possession of a complete and working invention. Dr. Alitalo tested the teachings of the opposed application and showed that the answer to this question is no.

VII. CONTINUED APPLICATION OF A SCIENTIFIC DOUBLE STANDARD BY HGS.

7.1 In Section III of OJB3, Dr. Ballard explains in detail one of the ironies of this opposition proceeding. The opposed patent application filed by HGS contains very little in the form of experimental or evidentiary support, and many of the experiments that were described therein have been shown to be defective, as explained in my first declaration. (See, e.g., OPR1 at 4.13-4.13.3.) Still, HGS's declarants seem perfectly content believing

every aspect of the opposed application, including aspects involving treatment of a huge variety of human diseases and ailments. At the same time that HGS's declarants adopt a position of faith with respect to the unsupported teachings of the opposed application, the same HGS declarants have urged that the Patent Office adopt very stringent criteria in this opposition proceeding for evaluation of scientific data presented by Ludwig Institute in its opposition. (See ANH2 and ASA2 declarations.)

7.2 Whether evaluating a patent application involving molecular biology or evaluating a sworn declaration involving molecular biology, a trained scientist should be applying the same scientific criteria. Scientifically, it makes no sense to believe one set of conclusions about VEGF2 that are unsupported by scientific evidence, even if they are written in a patent application, and to ignore scientific evidence that clearly contradicts the conclusions. Yet that is exactly what one finds upon examination of the HGS declarations. (See, e.g., OPR2, Appendix II. See also ANH2 and ASA2.)

7.3 For example, Dr. Hayward and Dr. Aaronson have urged that Dr. Alitalo's experiments should be given no weight, because of alleged defects in his selection or reporting of positive and negative controls. (See, e.g., ANH2 at 1.5; ASA2 at 5, 12, and 23.) However, the experiments reported in the opposed application clearly fail to report use of appropriate controls. (See OJB3 at 3.3-3.5) The statements in the opposed application regarding biological activity and treatment of diseases are unsupported by any reported experiments whatsoever, with or without controls. (OJB3 at 3.6) There is no scientific justification for placing faith in the teachings of the opposed application, especially when confronted with the evidence provided by Dr. Alitalo that the teachings in the opposed application are defective. Yet, that is exactly the position that the HGS declarants continue to adhere to. For example, Dr. Aaronson expresses the opinion that one familiar with the molecular biology of growth factors and "equipped with the HGS patent specification would recognize that the 350 amino acid polypeptide is a secreted growth factor . . . ." (ASA2 at 10.) Dr. Aaronson characterizes this as a "literal teaching of the HGS patent specification which describes VEGF-2 as a secreted growth factor . . . ." (ASA2 at 11.) These are, at best, statements of faith, because according to the scientific standards that Dr. Aaronson articulated for evaluating the Alitalo experiments, the skilled molecular biologist *would* conclude that the opposed application "fail[s] to provide any meaningful information regarding the expression, processing and secretion of VEGF-2" or its use as a "growth factor" for any purpose. (Compare ASA2 at 5 and 12.)

VIII. CONCLUDING REMARKS

8.1 Having examined the opposed application and all of the declarations that have been submitted by both parties, including the third and fourth Alitalo declarations, the following conclusions are self evident: (1) Dr. Power performed experiments involving VEGF2 subject matter, but her experiments were not based on, and did not test, the direct teachings in the application, i.e., whether VEGF2 of about 350 amino acids is expressed and secreted by cells. (2) Dr. Alitalo's experiments were based on these teachings of the opposed application, and they were refined over and over to address every criticism that a group of HGS experts could think of. Dr. Alitalo's results showed repeatedly that expression and secretion of VEGF2 does not occur in the manner taught by the opposed application. (3) HGS asked scientists other than Dr. Power to criticize Dr. Alitalo's work, but not actually run Dr. Alitalo's experiments themselves, or run any other experiments based on the explicit teachings of the application, or report the results of such tests. As a result, the only experimental evidence testing the teachings of the opposed application is the evidence reported by Dr. Alitalo.

AND I MAKE this solemn declaration by virtue of the Statutory Declarations Act 1959, and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DECLARED at Monash Medical Centre, V.

This 16 day of August, 2002

Peter Adrian Walton Rogers

Before me:

(Signature of Witness) (Medical Doctor)

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AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

STATUTORY DECLARATION

I, Kari Alitalo, a Research Professor of the Finnish Academy of Sciences, at The Molecular/Cancer Biology Laboratory, Biomedicum Helsinki, P.O.B. 63 (Haartmaninkatu 8) 00014 University of Helsinki, Finland. do solemnly and sincerely declare as follows:

I. INTRODUCTION

1.1 In July, 2002, I executed a third statutory declaration (hereinafter referred to as "OKA3" (Opponents, Kari Alitalo, 3rd Declaration)) to provide experimental evidence in support of the opposition filed by Ludwig Institute for Cancer Research ("Ludwig Institute") to the issuance of a patent to Human Genome Sciences, Inc., ("HGS") based on HGS's Australian Patent Application No. 696764 ("the opposed application"). A brief summary of the various declarations that have been filed throughout these proceedings was provided in OKA3 and will not be repeated here.

1.2 I hereby reaffirm my understanding that I have an overriding duty to the Patent Office (and to any Australian Federal Court that should review the Patent Office decision) to provide objective scientific analysis that I believe to be truthful. I hereby affirm that, to the

best of my knowledge and belief, factual statements herein are true and opinion statements herein represent my objective scientific opinion and analysis.

1.3 As described in OKA3, the purpose of my third statutory declaration was to design and perform protein expression experiments that would address any criticisms raised in the second series of declarations filed on behalf of HGS, particularly the second declarations of Dr. Nicholas Hayward (ANH2) and Dr. Stuart Aaronson (ASA2). Further, Ludwig Institute asked me to comment on the data from expression studies performed by Dr. Susan Power and reported in her second declaration (hereinafter "ASP2"), filed at the same time as ANH2 and ASA2.

1.4 The purpose of this declaration, hereinafter referred to as "OKA4", is to supplement the findings in OKA3 with an additional figure that depicts the results of transfection experiments for the Australian Patent Office. The results described in this declaration further demonstrate that the negative results are due to expression and secretion defects in VEGF-2 (as taught in the opposed application) rather than variations in transfection efficiency (as suggested, wrongly, by various HGS declarants). Also, I have attempted to further articulate the benefit of using two different transfection-efficiency controls (beta-galactosidase and luciferase) in OKA3 and again here in OKA4.

1.5 Although the experimental design reported in OKA3 and the experimental design of the present declaration are very similar, the protocol described below included an extra adjustment in immunoprecipitation sample sizes, to normalize for apparent modest experimental variation in transfection efficiency or other factors influencing the quantity of recombinant protein expression over time. (See paragraphs 4.1 - 5.5, below.) Other minor changes were made in the experimental protocol of the present declaration (e.g., times of incubations) due to time constraints and do not affect the results of the types of experiments performed and described herein. The experiments described herein provide further evidence that cells cannot express and secrete VEGF-2 as taught in the opposed application. On this point, the results were the same as the data already presented in OKA1, OKA2, and OKA3, further confirming that these declarations were accurate and were experimentally sound.

II. EXPERIMENTAL PROCEDURE

A. Cells and Plasmids

2.1 Results reported in OKA2 revealed that COS and 293T cells were equally appropriate cell lines for analyzing VEGF-2 protein expression and secretion. For these experiments, 293T cells were grown in DMEM supplemented with 10 % fetal bovine serum, glutamine and penicillin/streptomycin.

2.2 The polymerase chain reaction (PCR) was employed to construct a cDNA fragment that corresponded to amino acids 70 to 419 of prepro-VEGF-C. For the purpose of these experiments (directed to assessing transfection efficiency and protein expression at various time points) the cDNA fragment encoding amino acid residues 70 to 419 of prepro-VEGF-C corresponds appropriately with the cDNA encoding the full length sequence of the VEGF-2 polypeptide described in the opposed application. Nucleotides 559 to 1608 of the VEGF-C cDNA (Reported in Document D70, Joukov et. al. 1996, GenBank accession number X94216) were PCR amplified with the primers 5'-CGCGGATCCATGACTGTACTCTACCCA-3' containing a BamHI site and 5'-CGCTCTAGATCAAGCGTAGTCTGGGACGTCGTATGG-GTACTCGAGGCTCATTTGTGGTCT-3' containing a XhoI site, HA-tag, a stop codon and a XbaI site and cloned into pcDNA1(Amp)-vector (Invitrogen). The resultant vector was designated as VEGF-2(HGS)/pcDNA1.

2.3 An expression vector was also constructed that contained the full length (419 codons) VEGF-C sequence (OKA2 at 3.3.3) for use as a positive control in the expression and secretion analyses. The resultant vector was designated as VEGF-C/pcDNA1.

B. Transfection and time course:

2.4 A principle criticism alleged by the HGS experts was that my OKA2 declaration failed to include transfection efficiency data (ANH2 at 1.5; ASA2 at 24). Thus, for these new experiments, two separate expression vectors, pRL expressing Renilla Luciferase (Promega) and pCMV-x-gal expressing beta-galactosidase under CMV promoter, were used as transfection controls.

2.5 The other principle criticism of the procedures reported in my OKA2 declaration was regarding the lack of time points in the expression analyses (ANH2 at 1.5; ASA2

at 25). To address this concern, three different time points were tested in the new experiments. In particular, the 293T cells were split 1:6 and fresh medium was changed 19 hours thereafter. Three hours after medium change, VEGF-2(HGS)/pcDNA1, VEGF-C/pcDNA1, or empty vector were co-transfected with either pRL (three plates with each combination in a 16:1 ratio), or pCMV-x-gal (one plate with each combination in a 1:1 ratio), using FuGENE6 Transfection Reagent (Roche). The conditioned media and the cells were harvested 24 hours, 48 hours, or 74 hours after the transfection for the purpose of evaluating protein expression and secretion at these different time points. Either twenty-four hours (for time points 48h and 74h) or eight hours (for time point 24h) prior to harvesting, the cells were washed twice with PBS and changed to 3 ml of MEM medium containing 100 µCi/ml ^{35}S-methionine and ^{35}S-cysteine (Promix, Amersham) for metabolic labeling of proteins synthesized by the cells[1]. At the indicated time points the conditioned media was harvested and cleared by centrifugation. The cells were trypsinized, washed twice with PBS and lysed in 1x passive lysis buffer from the Dual-Luciferase Reporter Assay System (Promega).

C. **Beta-galactosidase staining:**

2.6 The cells were washed twice with PBS, fixed with 0.05% glutaraldehyde in PBS for 15 minutes at room temperature, washed three times with PBS, and stained over night with 2.5 mg/ml x-Gal (5-bromo-4-cloro-3-indolyl-ß-D-galactopyranoside) at 37°C. The beta galactosidase data provided evidence of transfection efficiency.

D. **Luciferase assay:**

2.7 The protein concentrations of the cell lysates were determined by using the BCA Protein Assay (Pierce). To quantify expression of recombinant protein in transfected cells, luciferase activity in cell lysates was measured with Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. The luciferase data provided evidence of transfection efficiency and recombinant protein expression efficiency at various time points.

[1] See explanation in OKA2 at 3.4.2

E. Immunoprecipitation:

2.8 Immunoprecipitation experiments were conducted to identify the presence of VEGF-C or VEGF-2 polypeptides in the conditioned media from the cells after the indicated incubation times.

2.9 For immunoprecipitation, aliquots of each conditioned medium were supplemented with BSA and Tween 20 to final concentrations of 0.5% and 0.02%, respectively. The different VEGF-2 or VEGF-C peptides were immunoprecipitated with polyclonal antibodies raised against a synthetic peptide corresponding to amino acid residues 104-120 of the VEGF-C prepropeptide (Antisera 882, reported in Document D71, Joukov et al., 1997) at 4 °C overnight. This peptide is present in the secreted form of VEGF-C, and the opposed application teaches that it should be present in mature VEGF-2 as well. Thus, antisera raised against this peptide should recognize VEGF-2 or VEGF-C polypeptides produced by the cells.

2.10 The immunocomplexes of secreted polypeptides bound to antisera 882 were precipitated with protein A-Sepharose for 2 hours and washed 2 times with 1X binding buffer (0.5% BSA, 0.02% Tween20 in PBS) and once with PBS at 4°C. The proteins were analyzed by SDS-PAGE in a 12 % gel under reducing conditions.

III. EXPERIMENTAL RESULTS AND ANALYSIS

A. Beta-galactosidase and transfection efficiency

3.1 In order to analyze transfection efficiency, the constructs of interest (VEGF-2(HGS)/pcDNA1, VEGF-C/pcDNA1, or the mock vector) were co-transfected into 293T cells with a plasmid encoding beta-galactosidase. Beta-galactosidase is an enzyme that causes production of a colorometric product, under the assay conditions used. In particular, successful transfection with the beta-galactosidase plasmid is observable as a colored colony on a culture plate. The presence and percentage of colored cells that are observable following the transfection experiments provides a measurement of relative transfection efficiency for the different transfections. HGS's declarant, Dr. Power, chose a beta galactosidase for her transfection efficiency control in her ASP2 declaration (See ASP2 at 20 and 24).

3.2 Ludwig Institute asked that I provide actual data in this declaration for the transfection efficiency study. Accordingly, I have included photographic results of the beta-

galactosidase staining as Figure 1B attached hereto (see **Exhibit KA-1** hereto). The figure represents photographs of culture plates following plating, transfection, and growth of the cells. Dark colored "spots" represent colonies formed from cells that were successfully transfected with the beta galactosidase vector and that are therefore expressing beta-galactosidase and appear dark blue under the assays conditions used. White or colorless cells, on the other hand, are not expressing the beta-galactosidase, and are scored as negative with respect to transfection.

3.3. The photographs in Figure 1B reveal that each of the transfections were very successful (numerous dark colored colonies were observed on all of the plates), and that the percentage of transfected cells were comparable in each of the three transfection groups (i.e., VEGF-2, VEGF-C, and mock). This data is sufficient to conclude that transfection efficiency is not confounding the results of my comparative expression studies, thus dispelling one of HGS's main criticisms of my OKA2 declaration.

B. Luciferase and Protein expression

4.1 The luciferase experiments provide a supplemental control for transfection efficiency that complements the beta-galactosidase study, and also provide an indication of the level of recombinant protein expression in transfected cells. The luciferase study was comparable to the beta-galactosidase study in the sense that it involved co-transfection of the constructs of interest (VEGF-2 or VEGF-C plasmid) with a second construct, the presence of which was measurable by a visualization assay. Because the co-transfected luciferase plasmid encodes an enzyme that causes production of light waves under the assay conditions that were used, the measurements of light units provides an indication that the transfections were successful. However, the luciferase assay differs from the beta-galactosidase assay because it quantitatively measures *recombinant protein* from transfection plates, whereas the beta galactosidase assay only measures the number of transfected versus non-transfected colonies, and does not provide a quantitative measurement of the amount of recombinant protein that the cells are producing.

4.2 The VEGF-2(HGS)/pcDNA1, VEGF-C/pcDNA1, or the mock vector were co-transfected into 293T cells with the plasmid encoding the Renilla luciferase gene as described above. At the datapoints selected for the experiment (24, 48, and 74 hours after transfection) conditioned medium was collected to assay for the secretion of VEGF-2 or VEGF-

C by the cells, and cell lysates were also analyzed to quantify the luciferase activity in the cells at the same time points.

4.3 The results of the luciferase assay are presented as a bar graph in Figure 1A (see **Exhibit KA-1** hereto), showing relative light units per microgram of protein for each of the three transfection groups at each of the time points specified. Since the luciferase assay is a measure of recombinant protein expression, it provides a second indication that both the VEGF-2 and the VEGF-C transfections were successful, because all groups showed luciferase activity at all time points. The fact that the relative levels of luciferase (in the VEGF-2 versus VEGF-C cultures) varies somewhat with time reflects the fact that the luciferase measurements are quantitative for the amount of recombinant, active luciferase protein present, rather than quantitative for percentage of cells successfully transfected. At all time points studied, the luciferase measurements in the VEGF-2 and VEGF-C plates were of the same order of magnitude.

C. **Analysis of VEGF-2 and VEGF-C expression and secretion.**

5.1 At the outset, it is my opinion that the differences in luciferase measurements between VEGF-2 and VEGF-C plates do not reflect a variable involving transfection efficiency that could account for the differences in VEGF-2 versus VEGF-C protein expression and secretion that I reported in all three of my previous declarations. In fact, the opposite is true. The luciferase data serves to validate the experimental design. The luciferase measurements were the same order of magnitude at each time point and indicate successful transfection of cells in both the VEGF-2 and the VEGF-C co-transfection experiments. The luciferase activity was abundant and measurable for both the VEGF-2 and VEGF-C co-transfections, whereas in the immunoprecipitation experiments, VEGF-2 has always been unmeasurable, while VEGF-C has always been easily measured. Thus, the transfection and cell culturing techniques are all sound. The "problem" is that the cells cannot express and secrete the VEGF-2 encoded by the VEGF-2 cDNA as taught in the opposed application.

5.2 Even so, for this declaration, I adjusted the immunoprecipitation experiments for *the benefit of the VEGF-2 transfections*, based on the luciferase data. Specifically, I presumed that the luciferase measurements provided an indication of the recombinant protein making capacity of the transfected cells. Based on this assumption, the

VEGF-C cells were making more recombinant protein than the VEGF-2 cells in these particular transfections. To compensate for the apparent difference, I used larger volumes of conditioned media from the VEGF-2 cells than from the VEGF-C cells for the immunoprecipitation. The volumes selected were as follows:

Culture Period	Volume of CM from cells transfected with VEGF-2(HGS)/pcDNA1	Volume of CM from cells transfected with VEGF-C/pcDNA1	Volume of CM from mock transfected cells
24 hours	690 microliters	450 microliters	1000 microliters
48 hours	235 microliters	125 microliters	1000 microliters
74 hours	265 microliters	180 microliters	1000 microliters

5.3. The relative amounts of conditioned media used were inversely proportional to the luciferase measurements. In each instance, more conditioned media from the VEGF-2 cells was used than from the VEGF-C cells to compensate for the lower production of recombinant (luciferase) protein in these cells. (If the VEGF-2 cells were secreting less protein per microliter of conditioned media, the use of larger sample sizes would compensate.)

5.4 No VEGF-2 protein was detected in the conditioned media from the cells transfected with the VEGF-2(HGS)/pcDNA1 construct at any of the time points tested, over a time period of 74 hours (Figure 2, Lanes 1, 4 and 7; see **Exhibit KA-2** hereto. The only bands visible in the VEGF-2 lanes of the gel are also visible in the "mock" lanes that have no VEGF-2 construct). In contrast, VEGF-C protein was effectively expressed and secreted by cells transfected with a vector encoding the full length VEGF-C. The different forms of VEGF-C immunoprecipitated from the conditioned medium correspond to partially and fully processed forms of VEGF-C (Figure 2, Lanes 2, 5 and 8. See bands corresponding approximately to the 30, 21.5, and 14.3 size markers). These results are in agreement with the results reported in OKA1, OKA2 and OKA3, and provide still further evidence that VEGF-2 as taught in the opposed application cannot be expressed and secreted by cells.

5.5 As Figure 2 shows, cells do not express and secrete VEGF-2, no matter what time point is used to terminate the experiment. Using extra conditioned media from the VEGF-2 cells fails to change the negative result. Expression of VEGF-C polypeptides, which serves as a positive control, was visible at all time points studied. VEGF-C expression was already visible at 24 hours and was strongly visible at 48 hours and 74 hours.

5.6 The extra experiments that I ran for OKA3 and this declaration demonstrate that all of HGS's criticisms of my earlier experiments were meritless. The protein expression and secretion data reported in OKA3 and the present declaration is essentially identical, indicating that transfection efficiency, time course, and any other miscellaneous factors causing minor fluctuations in recombinant protein production from one experiment to another do not alter the end result. The data reported herein confirms the conclusions of OKA1, OKA2, and OKA3, namely, that cells cannot express and secrete VEGF-2 as taught in the opposed application.

IV. CONCLUDING REMARKS

6.1 The protein expression and secretion studies I report herein were designed to address any criticisms made by HGS with regard to experimental design credibility. The results demonstrate several key points. First, VEGF-2 as taught in the opposed application cannot be expressed and secreted by cells. The data clearly establishes the failure of VEGF-2 to be expressed and secreted at multiple time points over a period of 74 hours (Each of these time points was sufficient to observe expression and secretion of the VEGF-C positive control run under the same experimental conditions). Second, transfection efficiency experiments reported herein rule out the possibility that absence of expression of VEGF-2 was due to insufficient amounts of the VEGF-2 expression vector being introduced into the cells. Even if the minor variations in transfection efficiency could contribute to variations in protein expression and secretion, this phenomenon was accounted for in the immunoprecipitation experiments reported herein by increasing the volumes of the VEGF-2 conditioned medium that were used in the immunoprecipitations. Thus, transfection efficiency, expression and secretion time course, and all other meaningful variables have all been accounted for in this declaration. This declaration,

and my three earlier declarations, all demonstrate that cells cannot express and secrete VEGF-2 as taught in the opposed application.

AND I MAKE this solemn declaration by virtue of the Statutory Declarations Act 1959, and subject to the penalties provided by that Act for the making of false statements in statutory declarations, conscientiously believing the statements contained in this declaration to be true in every particular.

DECLARED at Helsinki, Finland

This 14th day of August, 2002

Kari Alitalo

BEFORE ME

(Signature of Notary Public)
HANS SEVELIUS
Notary Public
14.08.2002

HELSINGIN MAISTRAATTI
MAGISTRATEN I HELSINGFORS

AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

THIS IS Exhibit KA-1

referred to in the Statutory Declaration

of Kari Alitalo

made before me

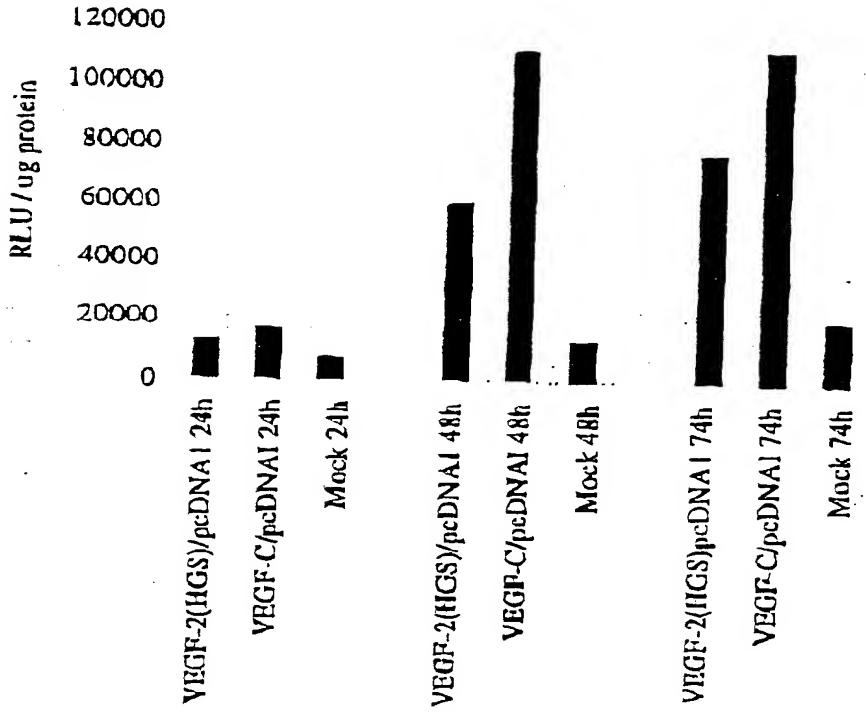
DATED this 14th Day of August 2002

(Signature of Notary Public)
HANS SEVELIUS
Notary Public

HELSINGIN MAISTRAATTI
HÖGSTRATEN I HELSINGFORS

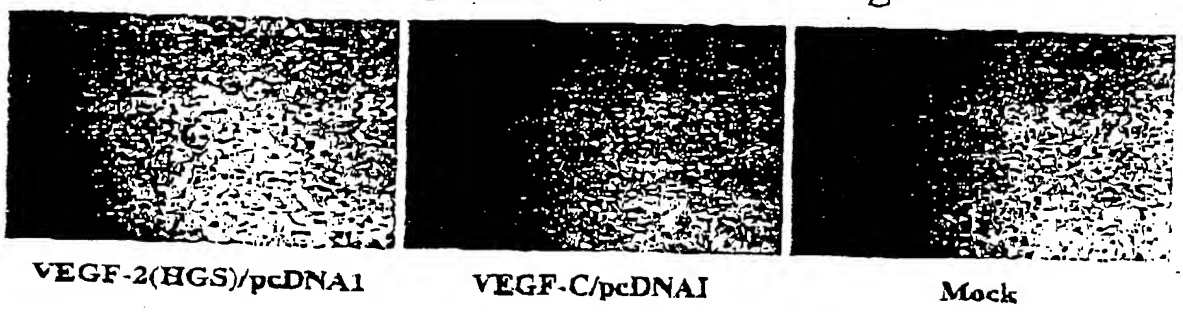
A

Renilla Luciferase Assay



B

Beta-galactosidase Staining



AUSTRALIA

Patents Act 1990

IN THE MATTER OF Australian Patent Application Serial No. 696764 by Human Genome Sciences, Inc.

-and-

IN THE MATTER OF Opposition thereto by Ludwig Institute for Cancer Research

THIS IS Exhibit KA-2

referred to in the Statutory Declaration

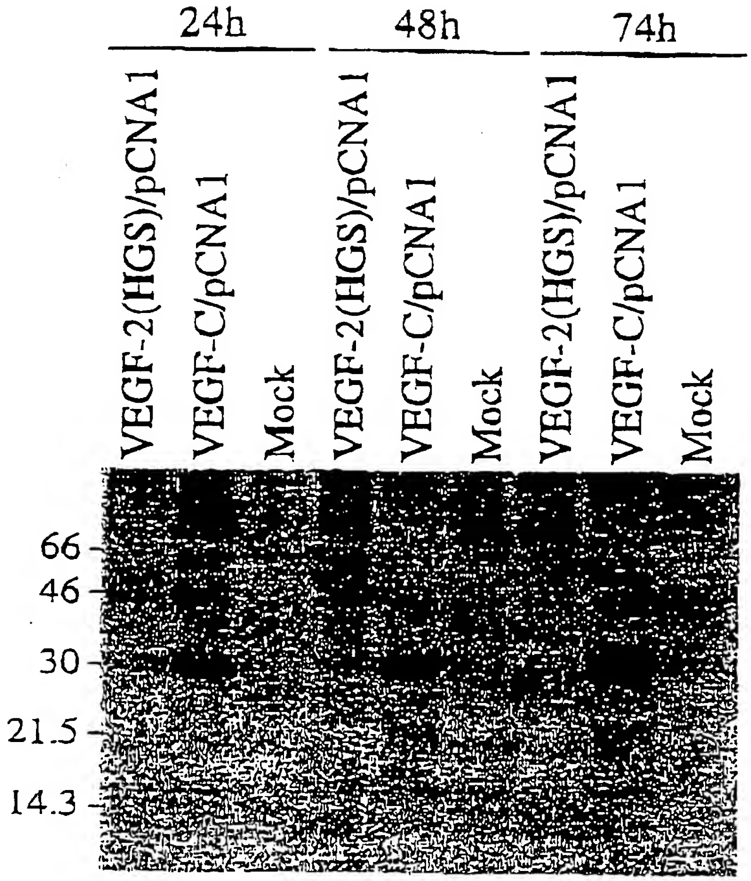
of Kari Alitalo

made before me

DATED this 14th Day of August 2002

(Signature of Notary Public)
HANS SEVELIUS
Notary Public

HELSINGIN MAISTRAATTI


24h
48h
74h
VEGF-2(HGS)/pCNA1
VEGF-C/pCNA1
Mock
VEGF-2(HGS)/pCNA1
VEGF-C/pCNA1
Mock
VEGF-2(HGS)/pCNA1
VEGF-C/pCNA1
Mock
66
46
30
21.5
14.3

Figure